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Simultaneous detection of cocaine and heroin metabolites in urine by solid-phase extraction and gas chromatography–mass spectrometry

Nadia De Giovanni*, Sabina Strano Rossi

Istituto di Medicina Legale, Universita' Cattolica del Sacro Cuore, L. go F. Vito 1, 00168 Rome, Italy

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Abstract

The present paper reports a method for the simultaneous extraction of cocaine, heroin and their metabolites from small amounts of urine (0.5 ml), using deuterated internal standards. Solid-phase extraction (SPE) on C_{18} columns followed by chromatographic separation coupled with mass spectrometry allowed the detection of all the substances after their derivatization. Mass spectrometry was performed in the electron-impact selected-ion monitoring (EI-SIM) mode. The limit of detection was found to be as low as 50 ng/ml for all the analytes; for reproducibility the C.V. was always better than 7%; the method was found to be linear with correlation coefficients between 0.989 and 1.00.

1. Introduction

The use of cocaine and heroin is the major drug abuse affecting our society today, and consequently their analysis in biological specimens has attracted great attention; moreover simultaneous consumption of cocaine and heroin is fairly common and undoubtely contributes to many drug-associated deaths.

Benzoylecgonine (BEG) and ecgonine methylester (EME) are two of the most important cocaine metabolites [1,2] and their simultaneous detection with cocaine itself is of great importance; morphine and 6-monoacetyl morphine (MAM) are the most important heroin metabolites [3–5], the occurrence of which is indicative of heroin abuse; moreover codeine (together with morphine) is often found in biological fluids after codeine ingestion [6]; sometimes codeine may also originate from the degradation of acetylcodeine frequently present in street heroin preparations.

The determination of these drugs is usually performed by immunoassay techniques [7,8] – which can only detect the primary metabolites and are often unable to discriminate between different substances – followed by chromatographic assays [9–11].

A more specific and sensitive technique involves gas chromatographic separation combined with mass detection, a confirmation analysis used for qualitative and quantitative purposes [6,12–15]. This kind of assay obviously requires a

* Corresponding author.

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preceeding extraction of the analytes from the biological fluids [12,16,17].

Because of their different polarities, simultaneous extraction and detection of cocaine, BEG and EME requires a time-consuming extraction procedure; moreover co-extraction of codeine and morphine can be problematic due to the amphoteric nature of morphine.

Cocaine in combination with heroin is one of the most frequently cited drugs combinations: hence the simultaneous assay of all their metabolites is important, but, however, still difficult, due to their different physical and chemical characteristics. This is demonstrated by the absence of data reported in the literature: only Darwin *et al.* [18] reported the simultaneous determination of cocaine and opiate metabolites in hair samples after solid-phase extraction (SPE). Other studies analysed either cocaine or heroin metabolites [12,13,19,20].

The present study proposes an easy and rapid solid-phase approach that allows the simultaneous extraction of all cocaine and heroin metabolites. The subsequent derivatization with bis-trimethylsilyltrifluoroacetamide gives the formation of their trimethylsilyl esters [16,21] which can be monitored by GC–MS using electron-impact selective-ion monitoring mass spectrometry (EI-SIM-MS).

2. Experimental

2.1. Reagents

All reagents and solvents were of analytical grade. Bakerbond SPE octadecyl 3-ml LD columns were obtained from J.T. Baker (Phillipsburg, NJ, USA). Deuterated cocaine, benzoylecgonine, morphine and monoacetylmorphine (internal standards) were purchased from Radian Corporation (Austin, TX, USA). Bis-trimethylsilyltrifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) was obtained from Sigma (St. Louis, MO, USA). Toxi-Tubes A and SPEC Toxi-Lab columns were from Toxi-Lab (Irvine, CA, USA). Extrelut high-volume extraction columns were purchased from E. Merck (Darmstadt, Germany). EME, cocaine, BEG, codeine, morphine and MAM were from S.A.L.A.R.S. (Como, Italy).

2.2. Sample preparation

Solutions of the deuterated internal standards were prepared in methanol with a concentration of 10 ng/μl. Aliquots (10 μl) of each standard were added to 1 ml of sample previously centrifuged at 3000 *g* for 10 min to give a final concentration in urine of 100 ng/ml. A 0.5-ml volume of the sample was then diluted with water (1:2) and extracted as follows: Bakerbond SPE 3-ml LD columns were flushed with 3 ml methanol and 3 ml phosphate buffer (0.1 *M* pH 7.5) under low vacuum, without allowing the column to dry. The pretreated sample was passed slowly through the column under vacuum and the column was rinsed with 3 ml of the same buffer. At this point the column was dried under vacuum for at least 2 min. Remaining water was removed with 100 μl of acetone. Elution was performed with 2 ml of chloroform–isopropanol (9:1, v/v). The eluate was dried under a stream of nitrogen and the residue was submitted to derivatization.

2.3. Derivatization

Derivatization was performed with 50 μl of BSTFA–1% TMCS at 120°C for 30 min. This step is necessary to convert the opiates to their trimethylsilyl derivatives; in contrast BEG and EME require only a flash-derivatization which can be performed by injecting 1 μl of BSTFA together with 1 μl of the mixture previously prepared; cocaine contains no active groups that can be derivatized with BSTFA.

2.4. Instrumentation

A Hewlett-Packard 5890 gas chromatograph with a 5971A mass-selective detector was used. A HP-1 capillary column (12 m × 0.2 mm I.D.; 0.33 μm film thickness) was used; the injector and interface temperatures were 250 and 290°C, respectively; the temperature program was as

follows: 120°C (held for 1 min) to 220°C at 20°C/min, then to 260°C at 5°C/min and finally to 280°C at 20°C/min and held for 2 min.

Mass spectrometry was performed in the electron-impact SIM mode. The ions monitored and their respective retention indices are as follows:

EME, *m/z* 96, 240, 271 (1582); Cocaine, *m/z* 182, 198, 303 (2146); Cocaine-D3, *m/z* 185, 201, 306 (2146); BEG, *m/z* 240, 256, 361 (2232); BEG-D3, *m/z* 243, 259, 364 (2232); Codeine, *m/z* 371, 178, 196 (2456); Morphine, *m/z* 236, 401, 429 (2518); Morphine-D3, *m/z* 239, 404, 432 (2518); MAM, *m/z* 340, 357, 399 (2557); MAM-D3, *m/z* 343, 360, 402 (2557).

3. Results

The proposed extraction method was compared with other techniques able to detect opiates and/or cocaine metabolites. The recoveries of the liquid–liquid extraction [pH 9.0 with chloroform–*tert.*-butanol (9:1, v/v)], obtained with Toxi-tubes A, Extrelut, SPEC Toxi-lab (as suggested by the respective manufacturers) and Bakerbond 3-ml LD, were determined for blank samples spiked with morphine. Table 1 reports the mean recoveries of each method: our extraction procedure showed better recoveries than the other methods; the same method successively applied for the extraction of cocaine, BEG and MAM gave mean recoveries of 92, 97 and 98%, respectively. Hence the proposed technique is able to extract simultaneously all the substances examined with recoveries better than 92% with good reproducibility (C.V. < 7%).

Table 1
Comparison of recoveries obtained from different extraction methods for morphine spiked samples ($n = 5$)

	Recovery range (%)	Mean recovery (%)
Toxi-tube A	27–35	31
SPEC Toxi-lab	23–64	41
Liquid/liquid	37–81	57
Extrelut	23–60	44
Bakerbond SPE	92–99	96

Several buffer solutions and elution solvents were tested: the choice of the optimum extraction conditions was made by looking for the best recoveries for all the substances (including cocaine and EME, which are instable at alkaline pH) with clean extracts. Drug-free samples, subjected to the method just described, gave no interferences when monitored in the SIM-mode.

Derivatization with BSTFA–1% TMCS resulted in the formation of trimethylsilyl derivatives of all the substances examined, except cocaine. Under the chromatographic conditions employed all the drugs could be separated. Fig. 1 shows a typical SIM chromatogram obtained from a real urine sample of a cocaine–heroin abuser: the presence of EME, BEG, cocaine, morphine, codeine and MAM is evident.

The sensitivity was found to be as low as 50 ng/ml for all substances. The linearity of the response was tested by analyzing a series of spiked urine samples: calibration curves constructed for concentrations between 50 and 500

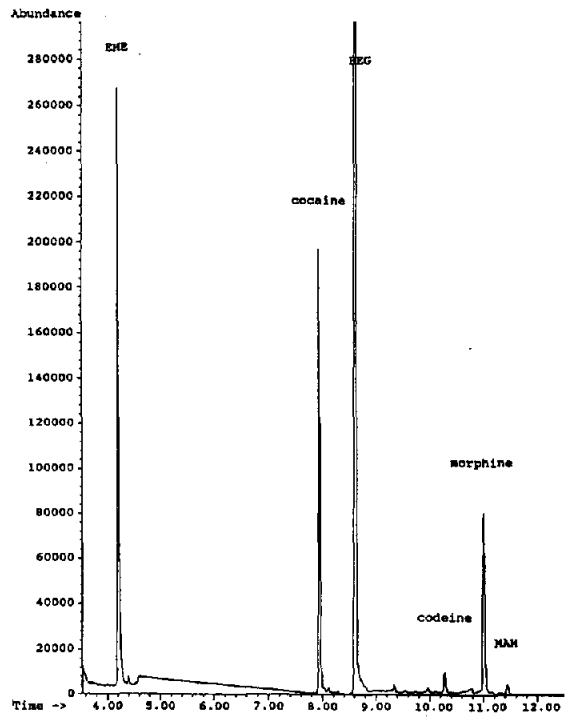

Fig. 1. GC–MS chromatogram (SIM mode) obtained after extraction of a cocaine–heroin abuser urine sample.

ng/ml for morphine, MAM and BEG, and between 50 and 2000 ng/ml for cocaine, gave good correlation coefficients (0.994 for cocaine, 0.989 for BEG, 1.000 for morphine, 0.991 for MAM). The accuracy of the method was acceptable with errors always lower than 10%.

Codeine and EME determinations were performed just for qualitative analysis to indicate their possible presence.

4. Discussion

As cocaine metabolism produces two major metabolites, BEG and EME, and cocaine itself is often present in urine (its concentration depending upon specimen pH), we consider it to be very important to identify and quantitate all these compounds: in particular, while BEG is normally used to demonstrate cocaine consumption, the detection of EME and cocaine can provide valuable and important forensic information.

In the same way the detection of codeine, morphine and MAM can give more information on heroin consumption. Moreover their simultaneous detection is useful in the quite frequently occurring cases of polydrug abuse.

The development of SPE allowed the isolation and purification of multiple analytes with substantially different chemical structures.

The proposed method is able to detect all the substances examined with good sensitivity (as low as 50 ng/ml): both the extraction and the chromatographic separation, quick and easy to perform, are suitable for their simultaneous determination.

Deuterium-labelled internal standards balance all possible analytical errors, such as incomplete extraction and/or derivatization.

The present method requires only a small amount of sample (0.5 ml diluted 1:2). However, the column capacity is higher, and by using a larger sample the sensitivity may be increased up to three times. Derivatization is necessary to convert the polar hydroxyl groups of the analytes into non-polar derivatives to improve their chromatographic behaviour and increase sensitivity. We chose silyl-derivatives because of their high stability [21].

The combination of SPE and the high sensitivity and selectivity of GC–MS resulted in an efficient assay for the detection of both cocaine and heroin metabolites in urine, helping to interpret the recent (ab)use of these drugs.

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References

[1] J.J. Ambre, T.I. Ruo, G.L. Smith, D. Backes and C.M. Smith, *J. Anal. Toxicol.*, 6 (1982) 26.
[2] J.J. Ambre, *J. Anal. Toxicol.*, 9 (1985) 241.
[3] E.L. Way and T.K. Adler, *Pharmacol. Rev.*, 12 (1960) 383.
[4] S.Y. Yeh, R.L. McQuinn and C.W. Gorodetzky, *J. Pharm. Sci.*, 66 (1977) 201.
[5] D.A. Smith and W.J. Cole, *Biochem. Pharmacol.*, 25 (1976) 367.
[6] E.J. Cone and W.D. Darwin, *J. Chromatogr.*, 580 (1992) 43.
[7] Abuscreen, Roche Diagnostics, Division of Hoffman-LaRoche, Nutley, NJ.
[8] EMIT Drug Abuse Urine Assays, Syva Corp., Palo Alto, CA.
[9] Toxi-Lab, Analytical Systems, Laguna Hills, CA.
[10] P.I. Jatlow, C. Van Dyke, P. Barash and R. Bych, *J. Chromatogr.*, 152 (1978) 115.
[11] N.C. Jain, D.M. Chinn, R.D. Budd, T.C. Sneath and W.J. Leung, *J. Forensic Sci.*, 22 (1977) 5.
[12] R.W. Taylor, N.C. Jain and M.P. George, *J. Anal. Toxicol.*, 11 (1987) 233.
[13] G.D. Clark, I.B. Rosenzweig, V.A. Raisys, C.M. Callahan, T.M. Grant and A.P. Streissguth, *J. Anal. Toxicol.*, 16 (1992) 261.
[14] D.M. Chinn, D.J. Crouch, M.A. Peat, B.S. Finkle and T.A. Jennison, *J. Anal. Toxicol.*, 4 (1980) 37.
[15] D.W. Hoyt, R.E. Finnigan, T. Nee, T.F. Shults and T.J. Butler, *J. Am. Med. Assoc.*, 258 (1987) 504.
[16] H.D. Rood, *Anal. Chim. Acta*, 236 (1990) 115.
[17] J. Scheurer and C.M. Moore, *J. Anal. Toxicol.*, 16 (1992) 264.

[18] W.D. Darwin, T.E. Maguire and E.J. Cone, *38th Annual Meeting of the CSFS Jointly with SOFT and IABPA*, Montreal, Sept. 23–27, 1991.
[19] W. Huang, W. Andollo and W.L. Hearn, *J. Anal. Toxicol.*, 16 (1992) 307.
[20] D.C. Fuller and W.H. Anderson, *J. Anal. Toxicol.*, 16 (1992) 315.
[21] B.H. Chen, E.H. Taylor and A.A. Pappas, *J. Anal. Toxicol.*, 14 (1990) 12.